THE ANAPHASE MOVEMENT OF CHROMOSOMES IN THE SPERMATOCYTES OF THE GRASSHOPPER

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Among the many complex processes involved in the division of cells, the movement of the chromosomes at anaphase is most accessible to a causal analysis. The beautiful preciseness of the processes involved in the orderly separation of chromosomes has for a long time enticed biologists to search for their physico-chemical basis. However, before an analysis on this level is possible, it is necessary, first, to know what structural differentiations of the cell are involved, and secondly, to have detailed quantitative descriptions of the processes based on a study of living cells.

A previous analysis of chromosome movement in certain insects (Homoptera and Hemiptera) has shown that the structures involved in anaphase movement are the kinetochores on the chromosomes, the chromosomal fibers, which connect the kinetochores to the spindle, and the spindle body (Ris, 1943). The kinetochore determines the nature of the chromosomal fibers, which in the case of these insects are broad and sheet-like and attached to the entire length of the chromosome (cf. Hughes-Schrader and Ris, 1941). The movement of the chromosomes consists of two separate processes: first, the shortening of the chromosomal fibers, which moves the chromosomes to the poles of the spindle; and secondly, the elongation of the spindle body, which further separates the chromosomes. In the Homoptera and Hemiptera these two components of anaphase movement are separated in time, so that first the chromosomes move to the poles of the spindle and then, after a pause of a few minutes, the spindle body stretches and carries the chromosomes further apart.

In most animals and plants the chromosomal fibers are narrow bundles attached to a definite, restricted region of the chromosome. In this paper the spermatocyte divisions of the grasshopper were chosen in order to analyze chromosome movement in an organism with localized kinetochore.

Material and Methods

The measurements recorded here were made on spermatocytes of *Chorthophaga viridifasciata*. A few measurements on *Dissosteira carolina, Melanoplus femur-rubrum, Arphia xanthoptera* and *Hippiscus spec.* gave similar results.

The spermatocytes of the grasshopper are classical material in the study of living cells in division (Chambers, 1914, 1924; Lewis and Robertson, 1916; Belar, 1929; Baumgartner and Payne, 1931). The usual technique consisted in breaking the testis follicles and spreading the cells on a coverglass in Locke's or Ringer's solution. Baumgartner and Payne (1931) showed that the follicles can be left intact and the cells studied with high powers. They pulled the testis through an opening

[1] Part of the work for this paper was done in the Department of Biology, Johns Hopkins University.

of the body wall into a little pool of salt solution, but left it attached to the vasa efferentia. Since they had to remove the follicular membrane which contains the trachae, the testes may as well be completely removed from the animal. In the present work the testes were dissected out, the follicular membrane removed, and the follicles spread intact on a coverglass into a drop of Belar's solution (Belar, 1929). The coverslip was inverted over a depression slide and sealed with paraffin. Aseptic technique was not attempted since only preparations made on the same day were used for measurements. The temperature was kept constant at 30° C. with an electric stage warmer. A liquid filter of ferrous ammonium sulphate prevented heat from the lamp from reaching the object. The cells, thus, were disturbed as little as possible.

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FIGURES 1 and 2. Camera lucida drawings of living spermatocytes of Chorthophaga. Side view of primary and secondary spermatocyte metaphases.

To measure the movement of chromosomes a metaphase in side view was selected, and as soon as the chromosomes began to separate, the distance between kinetochores was recorded at regular intervals with a camera lucida. In primary spermatocytes a bivalent with terminalized chiasmata near the spindle axis was chosen. In the secondary spermatocytes a chromosome in a median optical section of the spindle was selected. Though the spindle itself is hardly visible, it is clearly outlined by the chondriosomes (Figs. 1, 2). This makes it possible to measure the length and the equatorial diameter of the spindle during the entire anaphase. The various distances were then plotted against time, yielding a curve which describes the movement of the chromosomes and the changes in spindle length and diameter. All measurements were made with a 4 mm. Zeiss apochromat and 15 × ocular.

OBSERVATIONS

Anaphase movement in the first spermatocyte division

In Figure 3, three out of thirteen measured cells are presented.[2] The distance between separating kinetochores is plotted against time. The resulting curve con-

[2] The curves from different cells, even coming from different individuals, agree remarkably well, especially in the beginning of chromosome movement before spindle stretching sets in. The three cells shown indicate the degree of variation.

sists of an initial slow movement, then a straight portion of maximum velocity and a less regular part of gradually decreasing movement. Finally, before the cleavage furrow appears, the chromosomes move together again for a short distance, apparently due to the shrinkage of the spindle.

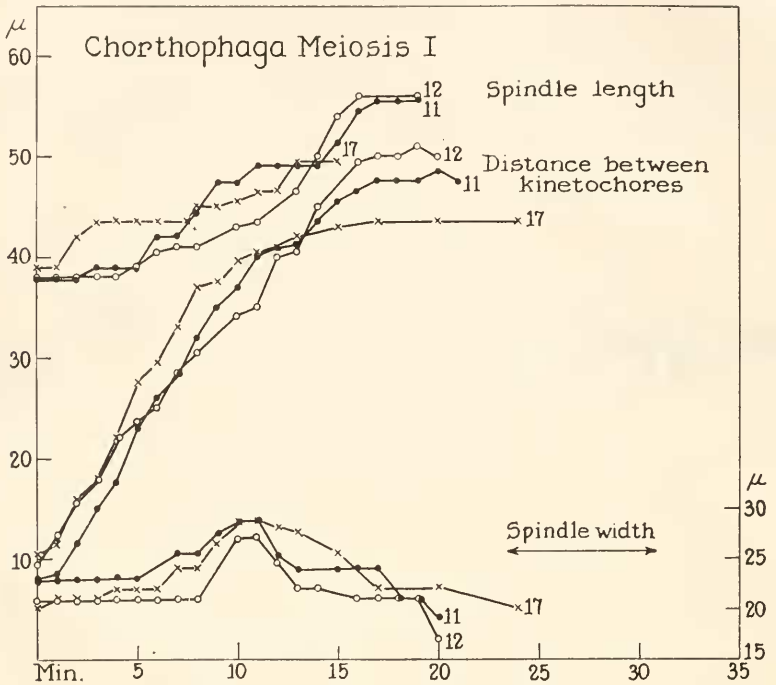


FIGURE 3. Chromosome movement and spindle behavior in the first meiotic division of *Chorthophaga viridifasciata*. See text.

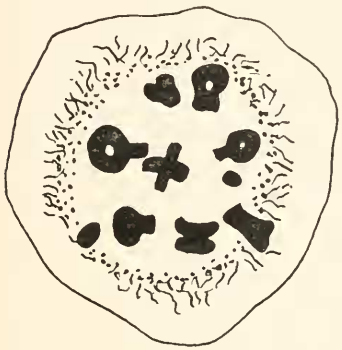
The spindle becomes visible in prometaphase through the alignment of the filamentous chondriosomes on its surface. In polar view their optical cross sections outline the spindle around its circumference (Fig. 4). In side view they appear lined up from the poles to the equator where they flow out into the equatorial plane (Fig. 1). Later, when the spindle elongates, the chondriosomes are stretched tightly on the spindle surface.

In Figure 3 the length of the spindle and its equatorial diameter are plotted against time during anaphase. We see how the spindle begins to elongate a few minutes after the onset of chromosome movement, contributing to their separation. There are thus two simultaneous processes involved in the later part of anaphase, namely, (1) the movement of the chromosomes to the poles due to the shortening of the chromosomal fibers, and (2) the elongation of the spindle. The diameter of the spindle increases in the later part of the chromosome curve, when the movement becomes irregular and slows down. Then it progressively decreases until the cleavage furrow cuts the spindle body in half. The spindle, therefore, increases

appreciably in volume during mid-anaphase. A comparison of the curves in Figure 3 shows that the spindle elongation varies more from cell to cell than the shortening of the chromosomal fibers. This indicates a greater sensitivity of that process to external conditions.

Anaphase movement in the second spermatocyte division

In the first division the chromosomes are distributed through the spindle body (Fig. 4). In the second division, however, they are oriented with their kinetochores at the periphery of a "hollow" spindle, the long arms pointing outwards (Fig. 5). Figure 6 gives the curves of three out of fifteen measured cells.[2] The



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FIGURES 4 and 5. Camera lucida drawings of living spermatocytes of Chorthophaga. Polar view of primary and secondary metaphases.

movements of the chromosomes and the behavior of the spindle are much like those described for the first division. The chromosome curve is distinctly S-shaped. The spindle elongates a few minutes after the chromosomes have separated and increases in diameter in the later part of anaphase. Again there is thus a great increase in volume of the spindle. The rate of chromosome movement and spindle stretching is appreciably greater than in the first division.

In both divisions then we find the same type of anaphase movement. It begins with a shortening of the chromosomal fibers moving the chromosomes towards the poles. While this is continuing, the spindle begins to stretch, adding to the chromosome movement. Towards the end of anaphase the spindle begins to increase in width and then gradually shrinks until the cleavage furrow cuts it in half.

Experimental separation of the factors of anaphase movement

In the Hemiptera and Homoptera the action of chromosomal fibers and spindle elongation represent two distinct processes separated in time (Ris, 1943). In the grasshopper there is no such independence; the two processes are simultaneous and so neatly interwoven that a smooth movement of the chromosomes ensues. Is it possible to separate them experimentally? Methods have long been known which inhibit or destroy the spindle, such as ether, chloralhydrate, colchicine, etc. Is

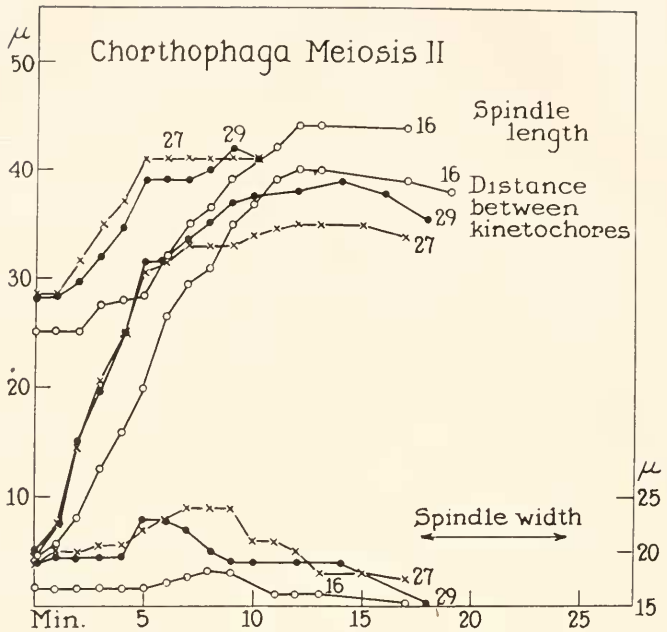


FIGURE 6. Chromosome movement and spindle behavior in the second meiotic division of *Chorthophaga viridifasciata*. See text.

there an agent which would inhibit one of the two processes without affecting the other? Colchicine, if added to the medium, either destroyed the spindle completely, or in lower concentrations had no effect on anaphase movement. Chloralhydrate, on the other hand, proved more useful. In concentrations higher than 0.1 per cent the spindle became shorter and narrower, and finally disappeared. The chromosomes were scattered irregularly through the center of the cell. The chondriosomes lost their regular orientation and began to penetrate between the chromosomes. At a concentration of 0.08 per cent cells were found in which the chromosomes moved to the poles, but where spindle elongation was inhibited. This seems to happen only within narrow limits of concentration of chloralhydrate inside the cell. If there is too much, the spindle will break down; if there is too little, it will elongate normally. This critical concentration is usually obtained only in a few cells of one cyst. The distance between chromosomes, and the length of the spindle, were then recorded during anaphase in primary spermatocytes exposed to chloralhydrate. In Figure 7 two such curves are shown (36 and 37). The spindle remained the same length all through anaphase in cell 37 and became only slightly longer in cell 36. The chromosomal fibers, on the other hand, must have remained active since the chromosomes had moved to the poles in a regular fashion. It can be shown that this action of the chromosomal fibers is normal. If we subtract the spindle elongation from the chromosome curve of an untreated primary spermatocyte, we obtain a curve which represents the movement of the chromosomes due to the chromosomal fibers alone. Two such curves are plotted in Figure 7 (13a and 17a). Since they agree well with the experimental curves (36 and 37), we must conclude that the

action of the chromosomal fibers was not affected by the chloralhydrate even though the spindle was prevented from elongating.

In the Hemiptera and Homoptera the two factors of anaphase movement, contraction of the chromosomal fibers and elongation of the spindle body, are separated in time. In the grasshopper they overlap in time, but their differing sensitivity to chloralhydrate has made it possible to separate them experimentally, inhibiting

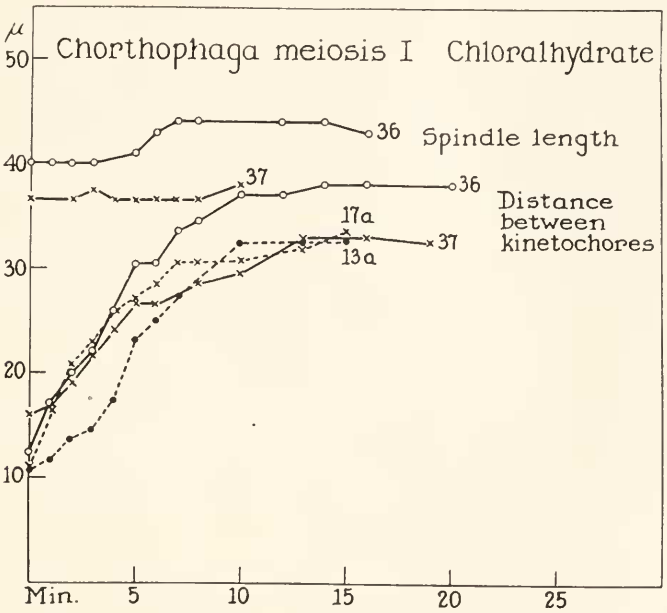


FIGURE 7. Chromosome movement and spindle elongation in Belar's solution with 0.08 per cent chloralhydrate (curves 36 and 37). Curves 13a and 17a represent the normal chromosome movement after the spindle elongation has been subtracted. The four curves are similar, showing that the movement to the poles in the absence of spindle stretching is normal.

spindle elongation without affecting the contraction of the chromosomal fibers. The difference between the grasshopper and the Hemiptera and Homoptera lies mainly in the relative timing of the component processes. In the grasshopper, spindle elongation sets in before the chromosomes have reached the poles. In the Hemiptera and Homoptera the spindle does not stretch until a few minutes after the poleward movement of the chromosomes has been completed.

Recently Callan (1941) described a case in which a separation of the components of anaphase movement occurs under natural conditions. In a trisomic grasshopper (Mecostethus) the unpaired extra chromosome sometimes moves into the equatorial plane during the first meiotic anaphase. In these cases the spindle does not elongate. The poleward movement of the chromosomes, however, does not seem to be disturbed. For reasons unknown, spindle elongation is inhibited under these conditions while the chromosomal fibers do not seem to be affected. Of course, we do not know here whether the rate of movement is normal as was shown in the chloralhydrate experiments.

The effect of temperature on the anaphase movement of chromosomes

The effect of temperature on mitosis has been repeatedly investigated, in most cases, however, on over-all processes such as the length of the mitotic phases, the rate of cleavage, etc. (see Belehradek, 1935). Only little can be concluded from such studies unless the processes are broken down into their components and the effect of temperature on these components analyzed. The effect of temperature on chromosome movement in living cells was studied by Bucciante (1927) in chick fibroblasts and by Barber (1939) in Tradescantia stamen hair cells. They found an increase in the rate of chromosome movement with rising temperature. This increase was large at lower temperatures and small at higher temperatures. In chick fibroblasts there is a maximum rate at 40° C. Fauré-Fremiet (1925) had earlier reported an optimum temperature (37° C.) for cell division in Ascaris. Measurements of chromosome movement in the secondary spermatocyte of the grasshopper at 17°, 23° and 30° C. agree with these findings (Fig. 8 and Table 1). The data for Tradescantia (Barber, 1939) and grasshopper also suggest an optimum temperature, though the range of temperature was not wide enough to show the decrease at higher temperatures. In the grasshopper a temperature above 32° C. destroys the spindle and thus inhibits chromosome movement.

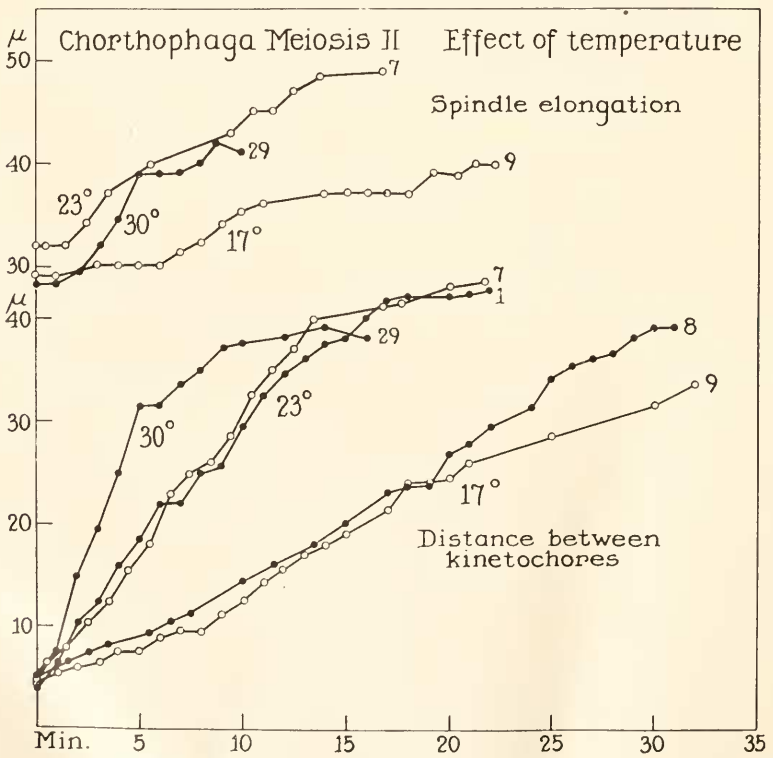


FIGURE 8. Chromosome movement and spindle elongation at 17°, 23°, and 30° C.

TABLE I

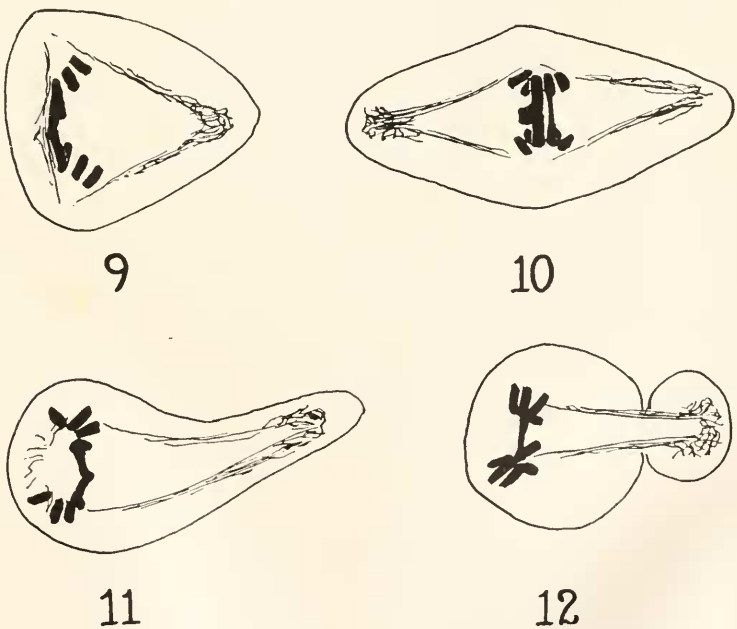
Effect of temperature on the rate of chromosome movement (chromosomal fibers only), and on the rate of spindle elongation. Micra/minute

Temperature	Maximum velocity chromosomes	Maximum rate spindle elongation
17° C.	0.4	1.4
23°	1.2	2.4
30°	2.5	3.6

Exposure to low temperature (1°–5° C.) destroys the mitotic spindle, as has been known since the experiments of O. Hertwig (1890) on sea urchins. If grasshopper spermatocytes at metaphase are exposed to 1° C., the spindle disappears, the chondriosomes become arranged at random, and the chromosomes are dispersed through the former spindle area. The cells can remain in this state for hours. If they are again exposed to a higher temperature (30° C.) the spindle forms anew, the chondriosomes are lined up on its surface and the chromosomes arranged in the metaphase plate. This process can be repeated several times on the same cells.

Abnormal spindle elongation

The increase in volume during anaphase is a characteristic property of the spindle in most animal cells. This swelling manifests itself especially in a pronounced elongation which contributes to the anaphase separation of the chromosomes that are attached to it by means of chromosomal fibers. As was shown above,



FIGURES 9–12. Diagrams demonstrating the abnormal lateral stretching of the spindle in primary spermatocytes after X-ray-induced sticking of the chromosomes.

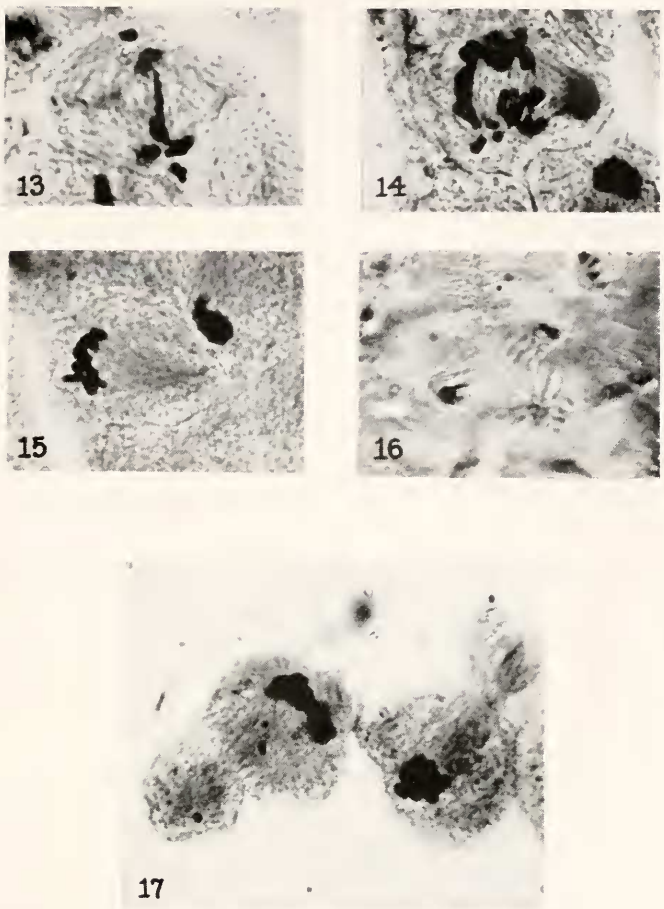
the spindle also increases in width in mid-anaphase and then gradually shrinks until it gets pinched through by the cleavage furrow. This stretching ability of the spindle is especially impressive under certain abnormal conditions. If the first meiotic division is observed in living cells after X-raying, in hypertonic medium or at temperatures around 32° C., one finds that chiasmata have a tendency to stick so that bivalents can not separate at anaphase. When the spindle begins to stretch, its normal elongation in the polar axis is inhibited by the combination of chromosomal fibers and sticking chromosomes. The spindle then begins to bulge in the equator, opposite the sticking chromosomes (Figs. 9, 14). If several bivalents fail to separate, the spindle may bend outward in several places (Figs. 10, 16). With the further elongation of the spindle, these lateral bulges become long and narrow projections which begin to push out the cell membrane. The poles of the spindle approach each other during this process, probably because of the action of the chromosomal fibers, which, instead of pulling the chromosomes to the poles, now draw the poles closer together (Figs. 11, 15). In fixed and stained preparations, the course of the continuous fibers shows clearly that the lateral projections are parts of the spindle bending outwards (Figs. 13–15). If chromosomes stick only on one side of the spindle, a very characteristic bent spindle results, looking like a spindle folded in the middle. Actually the origin is quite different, as described above. Sometimes the sticking chromosomes separate in mid-anaphase. The spindle then is able to assume its normal shape. It elongates in the polar axis and the lateral bulges disappear.

Spindle elongation and cleavage furrow

The relation of spindle elongation to cytoplasmic division, as demonstrated by these abnormal anaphases, is of special interest. When the spindle does not elongate normally, the cleavage furrow is always delayed or does not appear at all. More striking are the cells in which the spindle has been forced to elongate laterally in the equatorial plane. The lateral bulges of the spindle begin to push the cell out into long narrow processes (Figs. 11, 14). At the time when normally the cleavage furrow is formed, constrictions become visible around these cell projections. Often these constrictions develop into regular cleavage furrows and pinch off one or more small anuclear buds (Figs. 12, 17). In the cysts with secondary spermatocytes, one finds then cells with the diploid number of chromosomes which undergo the second division, and anuclear buds which do not divide any more. In grasshopper spermatocytes the cleavage furrow is therefore dependent on cell elongation caused by the stretching of the spindle. The location of the furrow is not predetermined, but can occur wherever the cell is pushed out.

Time relations

The main difficulty in the timing of the phases of mitosis, particularly in living cells, is the separation of the process into clearly delimited sections. The usual separation into prophase, metaphase, anaphase and telophase is not well suited for this purpose since the beginning or end of these phases is usually without sharp boundary. The duration of the following well-marked phases was measured in the spermatocytes of the grasshopper at 30° C.: First division: (1) Metakinesis, from



FIGURES 13–15. Anaphase in primary spermatocytes of Chorthophaga after irradiation with X-rays (100 r). Note the sticking of chromosomes and the lateral expansion of the spindle. Fixation: Sanfelice; stain: Iron-hematoxylin. 4 mm. Zeiss Apochromat, 15 × ocular. Compare with Figures 9–12.

the disappearance of the nuclear membrane to the formation of the metaphase plate. (2) Metaphase, from the establishment of the metaphase plate to the beginning of anaphase separation. (3) Ana-telophase, from the beginning of chromosome (kinetochore) movement to the appearance of the nuclear membrane. Interphase: from the formation of the nuclear membrane to its breakdown in the secondary spermatocyte. Second division: same phases as in the first division. The processes which mark these stages are clearly visible in the living cell. In the first division the asters are visible mainly due to the radial arrangement of the chondriosomes, but sometimes astral rays can be seen. The nuclear membrane, which was sharply outlined in prophase, becomes irregular and wrinkled, then disappears first near the asters. Wrinkled remnants can be seen for a few minutes before they vanish.

The metaphase spindle then slowly takes shape after the orientation of the asters. Its outline is marked by the chondriosomes. The spindle is at first rather narrow and short (cf. Belar, 1929, p 433). The chromosomes are thus crowded into the middle of the cell. Then, while the chromosomes become arranged into the metaphase plate, the spindle increases in width and in length. During metaphase the spindle remains constant in size and varies little from cell to cell. In telophase the nuclear membrane appears around a light area containing the chromosomes. The nucleus then enlarges until the regular interphase size is reached. In the second division the same processes are repeated, except that no asters can be seen in living cells. Table II gives the duration of these phases. Cells 1 and 2 were followed through the two divisions, cells 3 and 4 only through part of meiosis. Cells 3 and 4 are from a different individual than cells 1 and 2. At constant temperature the length of each phase varies only slightly from one individual to another.

TABLE II

Time relations in the meiotic divisions of the grasshopper Chorthophaga (30° C.) (hours and minutes)

	Cell 1	Cell 2	Cell 3	Cell 4
I. Metakinesis	50	—	43	—
I. Metaphase	2/30	2/45	2/40	—
I. Anaphase Telophase	1/25	1/40	1/25	—
Interphase	2/15	2/40	—	—
II. Metakinesis	25	—	—	25
II. Metaphase	1/25	1/25	—	1/35
II. Anaphase Telophase	1/45	1/47	—	1/45

DISCUSSION

The causal analysis of mitosis strives to dissect the complex process of cell division into its component factors and to elucidate their composition and their mode of action. Since Belar's classical study, the structures involved in the mitotic movements have again received deserved attention. In the grasshopper we can distinguish the following mitotic organelles: center, kinetochores, chromosomal fibers, spindle body.

The center

Like most animal cells the grasshopper spermatocytes contain a pair of centrioles which move to opposite sides of the nucleus in prometaphase and form the poles of the developing spindle. The asters are rather inconspicuous as in other cells with relatively little cytoplasm. In living cells they can be seen in prophase and occasionally in metaphase, especially in a hypertonic medium (cf. Belar, 1929). In secondary spermatocytes asters are even less distinct. Little is known about the function of the centers, except that they are probably involved in the organization of the spindle and in the cytoplasmic streaming which goes on during metaphase and anaphase.

Kinetochores

This specialized region of the chromosome is essential for the regular movements within the spindle. Fragments which are devoid of it lag behind and do not show any regular orientation, but may be moved passively by the stretching spindle or cytoplasmic currents on the surface of the spindle (White, 1935, 1937; Carlson, 1938). The main function of the kinetochores, perhaps in cooperation with the spindle or centriole, appears to be the formation of chromosomal fibers. Without kinetochores no chromosomal fibers can be formed.

Chromosomal fibers

Soon after the nuclear membrane has disappeared and the spindle begins to take shape, we can find in fixed and stained cells a distinct fibrous connection between the kinetochores and the spindle poles. These are the chromosomal fibers. They are usually not visible in living cells and some investigators therefore deny their existence.[3] Yet there is enough circumstantial evidence to show that they exist as differentiated structures within the spindle, and that they are the major factor in the anaphase movement of chromosomes (cf. Cornman, 1944; Schrader, 1944).

Belar (1929) emphasized the role of these "traction fibers." He assumes that they originate as a fluid secretion by which the kinetochore attaches itself to a fiber of the spindle body ("Leitfaser") and which allows the chromosome to glide along this "Leitfaser" in anaphase. Schrader (1944) accepts this view of Belar and bases on it his classification of spindles. Yet, the present writer could find no evidence for this indirect formation of the chromosomal fibers. They appear in prometaphase even before the spindle is fully formed as direct connections to the centers. They anchor the chromosomes to the poles of the spindle. So, when the spindle elongates, the chromosomes are carried along, the pull being transmitted through the chromosomal fibers to the kinetochores. If a chromosome sticks at anaphase, it will prevent the spindle from elongating on that side. The combination chromosome-chromosomal fibers is thus stronger than the spindle, while the cell membrane, for instance, yields to its pushing force. The chromosomal fibers, when they contract at anaphase, can even pull the spindle poles together and force the spindle out to one side of the cell (Figs. 9–12). The chromosomal fibers must thus be of greater consistency than the spindle body.

The spindle body

In grasshopper spermatocytes the spindle body develops from nuclear material between the two centers. The area around the chromosomes remains distinct even after the nuclear membrane has disappeared, and stays free of cytoplasmic inclusions like chondriosomes. In metaphase the spindle is a viscous body which can be moved about and dissected out by microneedles (Chambers, 1924). It appears homogenous in the living cell and fibrous after fixation. The spindle is essential for the orientation of chromosomes and for the action of chromosomal fibers since they are anchored at its poles. The spindle can be destroyed by a number of agents: colchi-

[3] Chromosomal fibers are sometimes visible in forms with diffuse kinetochore, if the chromosomes are viewed on end and the light therefore has to pass the entire length of the sheet-like chromosomal fibers (Hughes-Schrader and Ris, 1941; Ris, 1942).

cine, chloralhydrate, cold, heat, hypertonic medium, etc. At the same time the regular arrangement of the chromosomes disappears and all chromosome movements are stopped.

The most striking action of the spindle is the elongation during anaphase. Belar (1929) found that in hypertonic media this elongation appears to be greatly exaggerated, and this led him to a very detailed study of spindle stretching in hypertonic solutions. His conclusions are briefly: (1) The spindle has a tendency to stretch; this tendency is exaggerated in hypertonic media. (2) The spindle, by origin, is differentiated into two half spindles, the "Stemmkörper" (pushing body) developing at anaphase between the daughter plates. (3) At anaphase, it is the "Stemmkörper" in particular which elongates.

As was shown above, abnormal spindle stretching occurs not only in dehydrated cells, but always when daughter chromosomes are made to stick together. A hypertonic medium is just one way of causing chromosomes to stick at anaphase. This effect of hypertonic solutions on chromosomes was described by Konopacki (1911) in cleavage divisions of echinoderm eggs, by Kostanecki (1898) in Myzostoma, and by Moellendorff (1938) in tissue cultures. Similar accidents are found at high temperatures (over 30° C.) and after exposure to X-rays. It is therefore not the hypertonic medium which induces the abnormal spindle stretching, but the resistance to elongation in the main axis, brought about by the sticking of chromosomes. From Belar's figures, it is obvious that in primary spermatocytes all the abnormal spindles are correlated with sticking chromosomes. The bent spindles in secondary spermatocytes are of a different and less extreme kind (Fig. 45, Belar, 1929). Here it seems to be the cell membrane which offers resistance to the elongating spindle and causes it to bend. X-ray-induced bridges cause the same kind of abnormal spindles in secondary spermatocytes as Belar described in the first division. Lateral expansion of the spindle after X-ray-induced chromosome sticking was also figured by White (1937, Figs. 12, 13).

But Belar figures some cells which show exaggerated elongation of the spindle without sticking of chromosomes. These cells had been treated in anaphase. During anaphase the spindle increases not only in length, but also in volume. Belar believed that the volume remained constant, though he did not commit himself definitely. His beautiful drawings, however, indicate quite clearly the swelling of the spindle which measurements have now substantiated. In hypertonic solutions the cell shrinks greatly, and as Belar pointed out, the cytoplasm more so than the spindle. The swelling of the spindle, then, encounters resistance, and it is probably this factor which causes the spindle to be longer, but narrower than normally. Even so, these spindles are only found in free floating cells. In this writer's preparations where the cells remained in the follicles, they did not occur. Another case of spindle stretching without chromosome sticking is found if prometaphases are treated with hypertonic solutions (Belar, 1929, Fig. 55; Ris, 1942, in spermatocytes of the bearberry aphid). Here again the spindle increases in width during its formation. In dehydrated cells this lateral growth is interfered with, and the spindle becomes long and narrow. In this connection it is important to note that the volume of the abnormally stretched spindles appears to be not larger than in normal spindles. There is, therefore, only a distortion in shape, not an actual increase in the spindle material.

Two factors then cause abnormal spindles: interference with the increase in width in prometaphase and mid-anaphase, and interference with normal stretching during anaphase through the sticking of chromosomes.

From his studies of these abnormal spindles, Belar came to the conclusion that the part of the spindle between the separating chromosomes was mainly responsible for the stretching. He called it the "Stemmkörper" (pushing body) and distinguished it from the two half spindles between the chromosomes and poles. This subdivision of the spindle is, however, artificial and unjustified. Belar himself points out the uniformity in the aspect of the entire spindle. Fibers and clefts are continuous. The only difference in anaphase is the presence of chromosomal fibers in the cone-shaped region between the chromosomes and the poles. This is responsible for the darker appearance after staining. In Belar's Figure 41 the chromosomal fibers are especially clear. The "Stemmkörper" concept originated in the observation that the region between the daughter plates elongates more rapidly than the entire spindle. This appears so, not because this region is a special part of the spindle, but because the chromsomal fibers actually shorten during spindle elongation, pulling the chromosomes to the poles. In this way the impression of a special stem body between the daughter plates is produced.

Furthermore, Belar thought that the initial separation of the chromosomes through action of the traction fibers releases the tension in the spindle and originates the action of the "Stemmkörper." But actual timing has now shown that the chromosomes travel a good distance to the poles before the spindle elongates. Besides, spindle stretching can occur without any action of the chromosomal fibers as is shown in the first spermatocyte division of Tamalia. The chromosomal fibers act merely as passive anchors for the chromosomes (Ris, 1943). In the lepidopteran Orgyia the spindle elongates though the chromosomes have no chromosomal fibers at all (Cretschmar, 1928, Figs. 48–50). All the evidence then indicates that there is no differentiation into "half spindles" and "Stemmkörper." The only real differentiations are the chromosomal fibers and the spindle body. The chromosomal fibers pull the chromosomes to the poles. The stretching of the spindle has nothing to do with this phase. It can go on just as well without spindle elongation (chloralhydrate experiment). But spindle elongation has its important functions. It separates the daughter plates still further by pushing the poles apart and thus indirectly moves the chromosomes anchored to them.

The picture of anaphase movement in grasshopper spermatocytes presented here is essentially in agreement with Belar's view. There is a "pulling action" of chromosomal fibers and the stretching of the spindle. But there are some modifications. The subdivision into half spindles and "Stemmkörper" is found to be artificial. The spindle as a whole elongates, at the same time increasing in volume. Its action on the chromosomes is indirect, through the chromosomal fibers which connect them to the spindle poles.[4] The chromosomal fibers are thought to connect the kinetochores directly to the poles without the intervention of a "Leitfaser." They shorten during anaphase and are alone responsible for moving the chromosomes to the spindle poles.

[4] Just how the chromosomal fibers are attached to the spindle is a very puzzling problem and nothing definite can be said about it at present.

In addition to being a major factor in the movement of chromosomes, the spindle body also seems to play a role in the division of the cytoplasm. If the spindle does not elongate, as in the chloralhydrate experiments, no cleavage furrow is formed. When the spindle stretches laterally instead of in its long axis, a cleavage furrow does appear at a right angle to this elongation in a quite unorthodox position and produces an anuclear bud (Figs. 9–12). Bauer (1931) illustrates a similar situation in spermatocytes of Tipula with abnormal spindles. His Figure 23 h suggests that it originated in the same fashion. Many examples can be found in the literature which show how the failure of spindle stretching causes absence of the cleavage furrow (for instance Dobzhansky, 1934; Callan, 1941). In most plant cells there is little or no stretching of the spindle and the cytoplasm is divided by the formation of a cell plate. But in the pollen mother cells of some plants a cleavage furrow is formed, and it is then associated with elongation of the spindle (Guignard, 1897; Farr, 1918). It appears then that in dividing cells, elongation of the cell and cleavage furrow are associated with spindle elongation (in contradiction to the unwarrantable generalization of Buchsbaum and Williamson, 1943). Dan has recently (1943) assembled convincing evidence that spindle elongation is the active agent in cell elongation and the following formation of a cleavage furrow.[5] On the other hand, in certain abnormal cases the spindle elongates and yet no cleavage furrow appears. The formation of a cleavage furrow clearly depends on other factors in addition to spindle elongation.

In a recent paper, Hughes and Swann (1948) published chromosome separation and spindle elongation curves for chick embryo cells in tissue culture. Chick chromosomes possess a localized kinetochore and the achromatic apparatus is similar to that of the grasshopper spermatocytes. The anaphase movement as described by the curves of Hughes and Swann is essentially the same as we found in the grasshopper. Their curves show spindle elongation to start right from the beginning of anaphase, while in the grasshopper it does not begin until the chromosomes have moved a considerable distance. This is probably not a real difference but the result of the great difficulties involved in making measurements in early anaphase on the small chromosomes and spindles of the chick embryo cells.

We have set out to describe the movement of chromosomes during anaphase in terms of the mitotic organelles involved. The structures responsible for this chromosome movement were found to be the chromosomal fibers and the spindle body. The chromosomal fibers move the chromosomes to the spindle poles by decreasing in length. The spindle body swells and stretches and moves the daughter chromosomes further apart, since they are anchored to the spindle by the chromosomal fibers. If these are broad sheets attached to the entire length of the chromosome (diffuse kinetochore), the spindle does not elongate until the chromosomes have reached the spindle poles (hemipteran and homopteran insects). If the chromosomal fibers are narrow bundles attached to a very short region of the chromosome (localized kinetochore), the spindle begins to stretch shortly after the chromosomes have begun to move. The two processes then act simultaneously producing a smooth unbroken chromosome separation curve. Though we cannot see here directly how the two factors act on the chromosomes, we can separate them experi-

[5] I am indebted to Dr. D. Costello, University of North Carolina, for making this paper available to me.

mentally by inhibiting spindle elongation with chloralhydrate. It is then possible to study the action of the chromosomal fibers alone.

Very little is known about the nature and mode of action of these organelles, and this aspect will not be discussed here. Many more exact data on the structure, composition, and behavior of spindle and spindle components under various conditions are needed before a fruitful hypothesis on the physico-chemical level can be brought forward.

Summary

The movement of chromosomes and the changes in spindle size have been recorded in living spermatocytes of the grasshopper during the meiotic divisions. Anaphase movement consists of two separate processes which are related to the action of distinct cellular organelles: (1) The shortening of chromosomal fibers moves the chromosomes to the poles. (2) The elongation of the spindle further separates the daughter plates. The two processes act simultaneously in the grasshopper. With chloralhydrate, spindle elongation can be inhibited without affecting the action of the chromosomal fibers. This demonstrates the independence of these two factors.

The effect of temperature on chromosome movement is shown by measurements at 17°, 23° and 30° C. Between 17° and 23° there is a greater increase in velocity of chromosome movement than from 23°–30° C. Temperatures above 32° C. inhibit mitosis through the destruction of the spindle.

Abnormal spindle elongation is found whenever chromosomes stick at anaphase. The spindle, unable to elongate in its long axis, expands laterally into a disc-shaped body which later forms one or several finger-like processes, pushing out the cell membrane. These lateral elongations usually give rise to one or more cleavage furrows, pinching off one or, rarely, more anuclear buds. This demonstrates clearly the relationship between spindle elongation, cell elongation, and cleavage furrow.

The role of the mitotic organelles in the anaphase movement of chromosomes is discussed. Indispensable for a regular anaphase are the kinetochores on the chromosomes, the chromosomal fibers, and the spindle body. No evidence was found for a specialized region in the spindle acting as "Stemmkörper." The spindle is uniform in structure and elongates uniformly.

Distinct recognition of the structures involved in anaphase movement, and a quantitative description of their function, forms a basis for experimental analysis of their composition as well as their mode of action.

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